Michigan Department of Community Health
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Michigan Department of Community Health
Bureau of Laboratories

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Avian Influenza

Patricia A. Somsel, Dr.P.H.
Patricia A. Clark, M.P.H.
Division of Infectious Disease

The press has recently been filled with coverage of the spread of avian influenza among poultry in southeast and central Asia and of the associated human infections. After years of concern in public health circles for the inevitability of a pandemic, the highest levels of our nation's government have now recognized the potential for more widespread infection in humans and the impact such an outbreak would have on our country.

Influenza A virus is characterized by a system of typing two viral proteins, hemaglutinin (HA) and neuraminidase (NA). Human-to-human infections are associated with three of the 16 recognized influenza A hemaglutinin types (H1, H2 and H3) and with three of nine influenza A neuraminidase types. The subtypes that have been recognized to date in human-to-human infection have been H1N1, H1N2, H2N2 and H3N2.

While all known subtypes of influenza A virus can infect birds, there are three types of HA (H5, H7 and H9) which currently predominate in avian infections. Infections in both domestic and wild poultry by H5N1 are of the greatest concern because of demonstrated virulence in humans. Influenza viruses infecting birds can be of either a "low pathogenic" or "highly pathogenic" avian influenza (HPAI) nature, which describes the mortality of the avian hosts. These HPAI strains may lead to significant economic impact for the farmer, either through direct loss from infection, or through government-mandated destruction of flocks (de-population) to limit spread.

Avian influenza strains can infect humans and other mammals that have close contact with poultry. Initially these strains lack the genetics necessary for adaptation to human transmission, the infection is dead-end; not spreading to other humans. Infections are often severe or fatal.

When an avian influenza virus evolves the capability for human-to-human transmission, a new strain or antigenic shift emerges and circulates. Newly emerged strains are transmitted particularly widely because the human population lacks any antibodies. In essence, the entire current human population is at risk of infection due to immunologic naïveté.

Since 1997, newly emergent influenza subtypes infecting humans have occurred nine times: H5N1 in Hong Kong in 1997; H9N2 in China and Hong Kong in 1999; H7N2 in Virginia in 2002; H5N1 in China and Hong Kong in 2003; H7N7 in the Netherlands in

2003; H9N2 in Hong Kong in 2003; H7N2 in New York in 2003; H7N3 in Canada in 2004; H5N1 in Thailand, Vietnam, Indonesia, Russia, Kazakhstan, Mongolia, Malaysia, China, Cambodia, Turkey and Romania beginning in 2004 and continuing to the present. Severity of disease in humans varied with each subtype, but mortality rates of H5N1 infections have been upwards of 50%.

While the World Health Organization (WHO) seeks to detect and limit H5N1 outbreaks in poultry and to detect human cases worldwide, the Centers for Disease Control and Prevention (CDC) has enhanced surveillance for H5N1 avian influenza infection in the U.S. In Michigan, the Pandemic Influenza Plan, written over the past two years, has been released. Part of the plan involves clinical and public health microbiologists working together to identify, appropriately handle and rapidly transport specimens for testing.

Currently testing for avian influenza A (H5N1) is indicated for hospitalized patients with:

- Radiographically - confirmed pneumonia, acute respiratory distress syndrome (ARDS), or other severe respiratory illness for which an alternate diagnosis has not been established, **AND**
- History of travel within 10 days of symptom onset to a country with documented H5N1 avian influenza in poultry and/or humans (for a regularly updated listing of H5N1-affected countries, see the World Organisation for Animal Health (OIE) website at http://www.oie.int/eng/en_index.htm and the WHO web site at http://www.who.int/en/.)

Testing for avian influenza A (H5N1) is considered on a case-by-case basis in consultation with MDCH and the local health department for hospitalized or ambulatory patients with:

- Documented temperature of >38ºC (>100.4ºF), **AND**
- One or more of the following: cough, sore throat, shortness of breath, **AND**
- History of contact with poultry (e.g., visited a poultry farm, a household raising poultry, or a bird market) or a known or suspected human case of influenza A (H5N1) in an H5N1-affected country within 10 days of symptom onset.

According to federal legislation, HPAI H5N1 is classified as a Select Agent. Culture of clinical specimens for influenza A (H5N1) virus must be conducted under enhanced biosafety level (BSL) 3 conditions. These enhancements include controlled access double-door entry with change room and shower, use of respiratory protection (N95 or PAPR), decontamination of all wastes, and showering of all personnel upon leaving the BSL 3 facility. Labs working on or storing these viruses must be certified and registered by the United States Department of Agriculture (USDA).

Clinical specimens from suspect H5N1 cases can be tested by PCR assays under standard BSL 2 conditions in a Class II Biological safety cabinet (BSC). Commercial “rapid” antigen detection testing can be conducted under standard BSL 2 conditions, although there are indications that new biosafety guidelines will suggest use of a BSC for this testing. Unless it is BSL 3 enhanced and registered for select agents by the USDA, a clinical laboratory must not culture specimens for patients that meet the criteria above.

Initially, routine rapid influenza testing should be performed on-site. Once influenza A is determined through rapid testing and/or suspected H5N1 based on the above criteria, PCR confirmatory testing, available at MDCH, must be utilized rather than viral culture. Whether or not rapid testing is performed, once H5N1 is suspected based on the above criteria, PCR confirmatory testing must be utilized rather than viral culture.

Submit specimens from persons meeting the above clinical and epidemiologic criteria only after consultation with MDCH. Appropriate specimens include: bronchoalveolar lavage,

tracheal aspirates, and nasopharyngeal or oropharyngeal aspirates, washes or swabs. Nasal swabs are not acceptable specimens. Swab specimens must be collected using only Dacron tipped swabs with aluminum or plastic shafts. Specimens must be transported in viral transport medium. Specimens must be shipped to MDCH using the most rapid means available. Any cases of suspect H5N1 influenza must be communicated to MDCH or the local health department.

At MDCH, a PCR assay will be utilized to confirm the specimen contains influenza A virus. If influenza A positive, a second PCR assay will be performed to subtype the neuraminidase portion of the virus. If it is not N1, the specimen will be placed in culture for subtyping. If it is N1, suggesting the possibility of H5N1, the hemagglutinin portion of the virus will be subtyped by a third PCR assay. If non-H5, the specimen will be placed in culture for subtyping. If the specimen is positive for influenza A H5N1, it will be sent to CDC for further analysis. Culture will not be attempted at MDCH.

A human vaccine is under development for the H5N1 influenza virus with clinical trials now underway. In areas of the world with H5N1 cases, some officials recommend the use of current influenza vaccines to reduce the possibility of reassortment of H5N1 genetic components with those of current human-adapted strains, possibly resulting in a strain with enhanced human-to-human transmission.

Most H5N1 strains appear to be susceptible to oseltamivir and zanamavir but resistant to amantadine and rimanatadine. The latter two drugs are often used to prevent influenza in those who have been exposed to the virus.

Clinical laboratorians are at the interface of the community and emerging infectious disease, and are essential to the public health response to agents such as avian influenza. Their role is to be well informed, provide appropriate guidance to clinicians on specimen collection and handling, employ safe testing practices in the laboratory, and communicate with the local health department to prevent disease, promote wellness, and improve the quality of life.

For further information, check the MDCH website (www.michigan.gov/flu) or contact Patty Clark, Interim Virology Section Manager at (517) 335-8102 or clarkp@michigan.gov.

Influenza Isolate Submission

Patty Clark, M.P.H.
Virology Section

Laboratory based influenza surveillance monitors circulating viruses early and late in the season when disease activity is low; to monitor the severity of the season (the level of activity in the State); to identify strains circulating in early, mid and late season; and to guide vaccine strategy for the next season. In order to accomplish this last objective, the MDCH laboratory submits representative isolates to CDC three times per season (early, mid and late).

This year, CDC is requesting that the original specimen be submitted along with the viral isolate. If a clinical specimen is submitted to MDCH, it will be submitted to CDC with the isolate. When a lab submits an isolate to MDCH for confirmation, it is requested that the original clinical material be submitted at the same time. The clinical material can then be sent to CDC with the isolate.

CDC is requesting the original clinical material because, although isolates grow very well in tissue culture cell lines, they may not grow well in chicken embryos (the method of virus production for vaccines). By submitting the original material, CDC can culture an isolate in chicken embryos, to determine suitable strains for vaccine production.

Sottile Retires After 18 Years

Kirsten White, MT (ASCP)
Patricia Wheeler, BS
Upper Peninsula Regional Laboratory

On October 7, 2005, Dr. William S. Sottile left active service as the Laboratory Director of the Michigan Department of Community Health Upper Peninsula Regional Laboratory.

Sottile received his Bachelor of Science degree from Florida State University and his Doctorate of Philosophy from the University of Georgia. He became a Diplomate of the American Board of Medical Microbiology in 1985. Prior to his state service, he was the Director of Clinical Microbiology Laboratory Services for Chicago College of Osteopathic Medicine.

Dr. Sottile was the director of the U.P. Laboratory in Houghton from 1987 to 2005. He was responsible for directing staff and managing the laboratory, conforming the laboratory to CLIA standards for clinical testing and EPA standards for water testing and was the Coordinator of the MDCH Michigan Regional Laboratory System.

Sottile directed the laboratory, as it became a Level B, Reference Laboratory, in the Centers for Control and Preventions' Laboratory Response Network (LRN) and initiated statewide molecular epidemiology testing to assist healthcare facilities in nosocomial outbreak investigations. He also led 23 local public health facilities in Michigan in clinical testing under a single CLIA certificate. During his state tenure, Sottile held an adjunct appointment as Professor at Michigan Technological University where he taught a course in Medical Bacteriology to students in the medical technology and pre-med programs.

Under Sottile's direction, the U.P. Laboratory moved in December of 2004. It is now located in a new facility in the Advanced Technical Development Center of Michigan Technological University on Sharon Avenue in Houghton (see *LabLink*, Vol. 10, no. 3). The new facility brings Biosafety level (BSL) 3 capabilities to the laboratory maintaining its status as a reference laboratory in the LRN.

Dr. Sottile was an active member of the Michigan Society for Infection Control and Prevention (MSIC) from 1989 to 1998. During much of that time, he served on the governing board He was responsible for converting the MSIC database from a card-based system to a computer database. During his term as MSIC Membership Chair, a member directory was published to facilitate networking among the membership. After leaving active membership with MSIC, he continued to consult on development of the membership database and its conversion into MS Access.

Dr. Sottile is now looking forward to summers in Michigan, winters in south Florida, gardening, boating and fishing and spending time with his family. We will miss Dr. Sottile, and wish him much happiness in his retirement!

How Is Our Service?

Does your facility use the laboratory services at Michigan Department of Community Health? Please take a few minutes to tell us how we are doing!

Our on-line survey is available at http://www.questionpro.com/akira/TakeSurvey?id=283360 or email us at sasyn@michigan.gov and we will send you a link to the survey. If you would prefer a hard copy of the survey to fax back to us, please call Martha Boehme at 517-335-9654.

Filling out the survey will only take five minutes of your time and the information collected is invaluable to the Bureau of Laboratories. The results from this survey will be used to draft priorities for improving services as part of the laboratory strategic planning process. We ask that you please respond by **December 30**. Thank you for your input!

Changes in MDCH Hepatitis C Algorithm

Patty Clark, M.P.H.
Virology Section

The Centers for Diseases Control and Prevention (CDC) published its "Guidelines for Laboratory Testing and Result Reporting of Antibody to Hepatitis C Virus" in the February 7, 2003, MMWR, Vol. 52, No. RR-3.* Shortly thereafter, the MDCH Virology laboratory altered its Hepatitis C Virus (HCV) test algorithm to comply with these recommendations. The algorithm used the anti-HCV EIA as a screening assay. Repeatedly reactive (RR) anti-HCV EIAs were sorted by serum to cut-off (s/co) ratios to determine the need for supplemental testing. Those RR samples with high s/co ratios (= 3.8) were reported without supplemental testing. Those RR samples with low s/co ratios, < 3.8, received HCV PCR testing. If the sample was HCV PCR negative or indeterminate, a RIBA strip immunoassay was performed. The PCR was selected as the first supplemental test because, at MDCH, PCR is less expensive to run than the RIBA.

Since 2003, the MDCH laboratory has generated much data surrounding this algorithm. Reviewing this data, it was found that almost all samples tested by the HCV PCR required additional RIBA testing. Doing a cost analysis on a recent subset of samples indicated a substantial cost savings would be achieved by removing the PCR assay from the algorithm. This change would also decrease specimen turn around time by approximately one week.

The new MDCH algorithm still follows the CDC guidelines, however, repeatedly reactive HCV EIA samples with low s/co ratios are reflexed to supplemental RIBA testing. Testing stops with the RIBA result. HCV PCR will not be available. A flow diagram of the new HCV test algorithm can be found at:

http://www.michigan.gov/documents/HCValgofig_70102_7.pdf

If you have questions regarding this change or any other aspect of HCV testing, please contact Patty Clark at (517) 335-8102.

* A complete copy of the Guidelines is available at:
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5203a1.htm

Planning For the Future

John Dyke, Ph.D.
Bureau of Laboratories

The world of public health has seen dramatic changes over the last several years. These changes have challenged traditional thinking regarding the roles and responsibilities of public health laboratories. Factors such as globalization of trade, the reduction of health care dollars, personnel shortages and most recently, natural disasters have created strains on public health resources. However, they have also created opportunities for self-examination.

The Michigan Department of Community Health, Bureau of Laboratories, like many of its community partners, is currently engaged in organizational planning. The outcome of this planning will help develop future strategies combined with operational effectiveness to provide the highest quality of services to the citizens of Michigan.

The Bureau cannot accomplish this goal alone. Throughout the planning process, the assistance of both internal and external partners will be essential for ultimate success. MDCH values partner in-put and looks forward to building a shared vision and meeting the needs of all concerned with public health in Michigan.

Chemistry and Toxicology Update

Kevin Cavanagh, Ph.D., FACB
Division Director

Trace Metals Section

The trace metals section received accreditation from American Industrial Hygiene Association (AIHA) for another two years. This is a very rigorous evaluation of the environmental lead laboratory. Congratulations to Jeff Dupler, Cheryl Laviere and the entire trace metals section for this major accomplishment.

Effective September 1, the trace metals section has begun accepting filter paper specimens for blood lead. For questions about collection kits, please contact the clinical specimen shipping unit by phone at 517-335-9867 or by fax at 517-335-9039.

Newborn Screening Section

At the National Newborn Screening Meeting held on October 24 – 27 in Portland, Oregon, two papers from the Michigan Newborn Screening (NBS) Program were presented.

Dr. Bill Young presented "Evaluation of the Screening Algorithm for Maple Syrup Urine Disease in Michigan." Co authors were Karen Andruszewski, Denise Pleger, Caron Burns, Eleanor, Stanley, Harry Hawkins, Kevin Cavanagh, Robert Grier, and Ayesha Ahmad.

Dr. Bob Grier presented "Age-Related Cutoffs for Newborn Screening by Tandem Mass Spectrometry." Co-authors were Caron Burns, Eleanor Stanley, Pat Garrod, Harry Hawkins, Kevin Cavanagh, and William Young.

During the previous *LabLink* report (see *LabLink,* Vol. 10, No. 2), it was announced that a pilot study for expanded newborn screening that complies with the American College of Medical Genetics guidelines (http://mchb.hrsa.gov/screening/) had been started. This pilot study remains in effect screening for 28 primary conditions.

In a related matter, Senate Bill 794 introduced by Senator Tom George, (R-Kalamazoo) provides for an Advisory Committee to be established to review the standard of care and to make recommendations on the NBS test menu and fee structure. For questions on the Newborn Screening Program, please contact the NBS office at 517-335-9205.

Analytical Chemistry Section

There continues to be a great deal of activity towards preparedness. The section's laboratory method for Nitrogen Mustard metabolites (random urine) was validated by the CDC. This group of analytes is now officially a part of the laboratory preparedness chemical menu.

The Bureau of Laboratories hosted a meeting with the Michigan American Association of Clinical Chemistry on Sept 22, 2005. Dr. Dick Scheel gave the scientific presentation "Michigan Preparedness Update for the Chemical Terrorism Laboratory Network: Analysis of Isocyanates and other Toxic Industrial Chemicals in Biological Fluids."

Dr. Paul Loconto presented at the Anachem/SAS Symposium in Livonia, Michigan on Nov 10, 2005. His talk provided an update on the section's activities including fish testing, chemical terrorism, PBDEs, white powders and analytical chemistry.

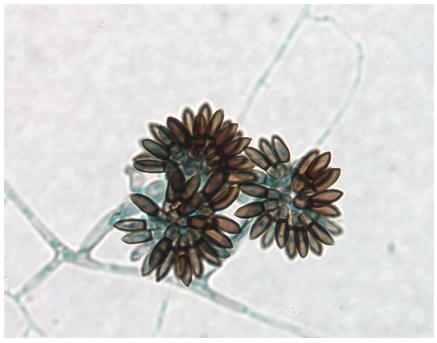
For questions about Chemical Terrorism Laboratory Education Programs, please contact Ninah Sasy at (sasyn@michigan.gov) or call 517-335-9152.

FUN FUNGI.....
Picture Quiz Review

Sandy Arduin MT(ASCP) & Bruce Palma MT(ASCP) - Mycobacteriology/Mycology Unit

Picture Quiz Review: How many do you remember?

1.



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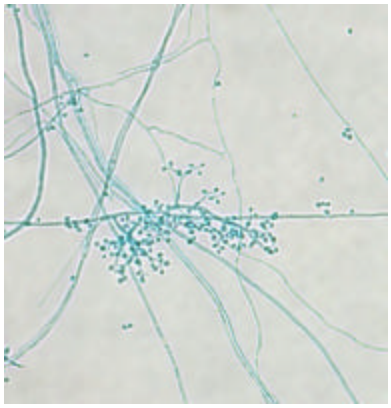

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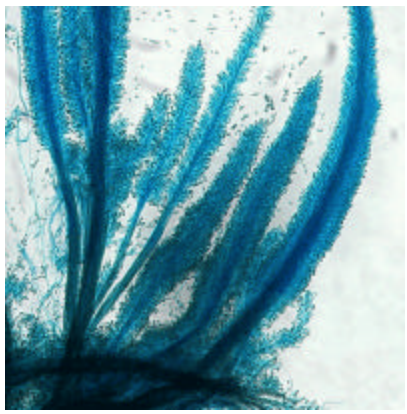

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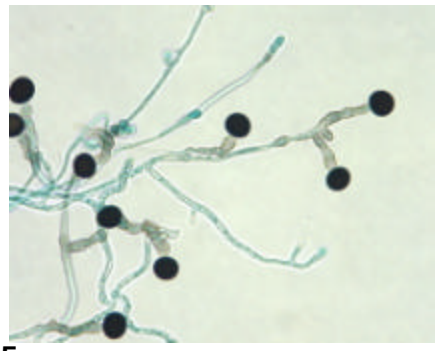

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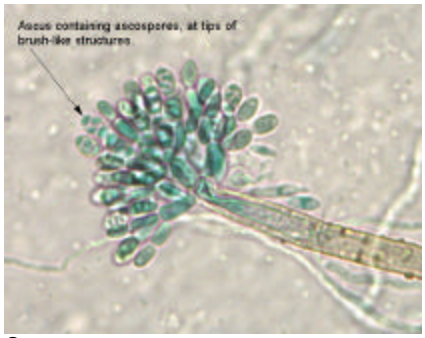

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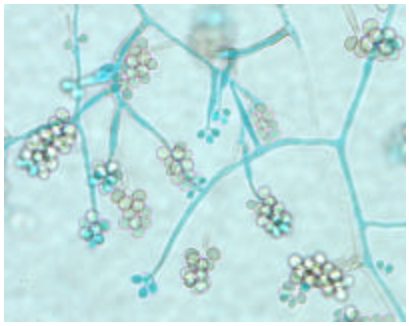
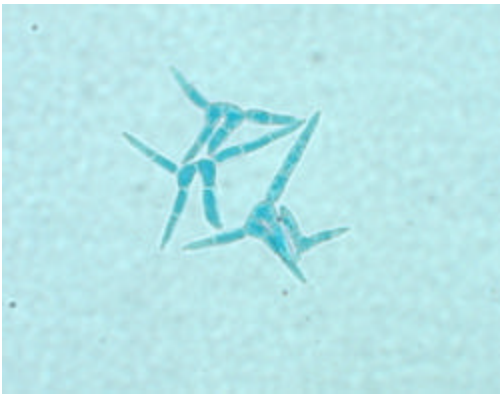


7.

Last Issue=s Picture Quiz Answer:
Tripospermum species



Tripospermum species are commonly found on leaves, twigs, and deciduous plants in tropical and temperate regions. Microscopically, conidiophores are inconspicuous and differ little from the vegetative hyphae. Conidia (staurospores) are hyaline to dark brown and have up to five arms. Typically the staurospores consist of two pairs of divergent, septate arms that arise from two connected basal cells.

Picture Quiz Review Answers:

1. ***Wardomyces* spp.:** The sporogenous cells bear apical clusters of dark ellipsoidal conidia. The conidiogenous cells are globose to barrel shaped and produce several conidia. The conidia are generally 2-celled and constricted at the septum. The apical cell is twice as long as the basal cell.

2. ***Beauveria* spp.:** The conidiogenous cells have inflated bases and terminate in a rachis (zigzag filament). Conidiogenous cells are often grouped in clusters. Conidia are hyaline and round to oval in shape.

3. ***Cephalotrichum* spp.:** Dematiaceous conidiophores form a synnemata with a fertile spore bearing head. The head is elongate and feathery, comprised of a central axis of hyphae, which bears numerous annellophores. The annellophores are short and inflated. They sometimes produce long chains of spores.

4. ***Nigrospora* spp:** The conidiophores are short and inflated and are typically hyaline but may be slightly pigmented. Conidia are black, unicellular, ovoid to ellipsoidal and oblate (slightly, horizontally flattened). Conidia also have a thin, equatorial germ slit, which is easier to visualize when the colony is young.

5. ***Cephaloascus fragrans*:** This is the only member of the family Cephaloascaceae. It resembles the yeast-like fungi of the Endomycetaceae family. It differs from others in that it produces 4, rarely 8, hat shaped ascospores in asci, which are formed by budding at the tip of an ascophore.

6. ***Tritirachium* spp.** The conidiophores are long, narrow and hyaline. The upper part of the conidiophore bears conidiogenous cells with a swollen base that taper to a sympodial rachis. They are arranged in verticilliate whorls. Conidia are globose, hyaline to golden in color and are produced in acropetal succession on the rachis-like sporogenous cell.

7. ***Pestalotia* spp.:** *Pestalotia* spp. are characterized by the production of acervulus (a flat or saucer-shaped stroma or hyphal mat containing a stand of closely-packed conidiophores) bearing dark phragmospores (multi-celled spores with transverse septa). These phragmospores have hyaline end cells each bearing two or more setulae (a delicate, hair-like, non-motile appendage found at the ends of the conidia).

Massey on the Move

Patricia A. Somsel, Dr.P.H.
Division of Infectious Disease

It is our pleasure to announce that Dr. Jeffrey Massey has taken an assignment as interim Director of the Michigan State Public Health Laboratory in Houghton. If you know Jeff well, you know that is a dream-come-true for him. He has always enjoyed the Houghton – Hancock area and is delighted to return to the home of his *alma mater*, Michigan Technological University.

Molecular biology testing will be integrated into the microbiology and virology sections. This integration is appropriate to the evolution of molecular techniques from a developmental stage in the demonstration of agents to well-accepted, standardized test methods routinely utilized in public health and clinical laboratories alike.

The National TB Genotyping Project and PulseNet work, previously under Dr. Massey's purview, will now be overseen by Dr. James Rudrik and his staff in the Microbiology Section. Viral PCR and sequencing, including HIV genotyping, will be transferred to the Virology Section, currently under the direction of Patty Clark.

Please join us in congratulating Dr. Massey on his new assignment.

Loconto Releases Second Edition

Taking readers from the fundamental principles to state of the art methods, the *Trace Environmental Quantitative Analysis: Principles, Techniques and Applications*, Second Edition by Paul R. Loconto, Ph.D. was released on August 29, 2005.

This edition reflects the most recent advances in trace environmental quantitative analysis (TEQA). It extends the practice of TEQA to those analytical chemists involved in biomonitoring or chemical terrorism preparedness.

Loconto is a clinical health scientist at MDCH in the chemical analysis section of the Division of Chemistry and Toxicology.

Remember to Bookmark These Important MDCH Websites:

Bureau of Laboratories website at:
www.michigan.gov/mdchlab

Emerging Infectious Disease website at:
www.michigan.gov/emergingdiseases

Laboratory Training Calendar website at:
http://mdch.train.org/calendar/lab/calendar.htm

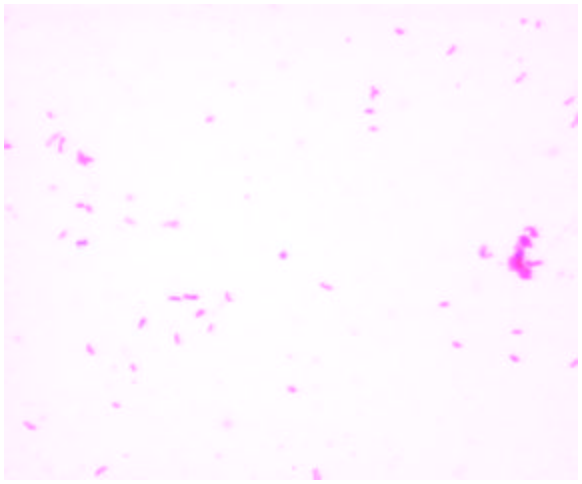
MI-TRAIN Learning Management System at:
http://mi.train.org

Quirky Bugs…..

Stephen Haskell, BS, SM(ASCP)
Reference Bacteriology Unit

Frequently, the reference bacteriology unit at the MDCH laboratories receives rare and unusual bacteria for identification. One such bacterium submitted was from the stool of a 46-year-old male who had developed diarrhea shortly after returning home to Southeastern Michigan after a Gulf Coast trip. Clinical history revealed that the patient had eaten raw oysters before his return to Michigan.

A lactose non-fermenting colony was isolated on the MacConkey agar from his stool culture. Gram stain showed a slightly curved short gram-negative rod. Preliminary testing indicated that this organism was oxidase positive, catalase positive, motile and fermented glucose without gas production when grown on a TSI slant. The colonies were characteristically beta hemolytic, convex, and had entire margins on 5% sheep blood agar plate. A presumptive identification placed the organism in the *Vibrio* genus.



Gram stain (1500X)

24hours on 5% Sheep Blood

The isolate was tested with a battery of bio-chemical tests designed to speciate members of the *Vibrio* genus. The isolate was also plated to thiosulfate-citrate-bile salts-sucrose agar medium (TCBS). All test media were incubated for 7 days at 37° C in ambient atmosphere. The results are listed below in Table 1. Both serology agglutination tests for *V. cholerae* serogroup O1 and O139 antigens were negative.

Three *Vibrio* species, *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* are well-documented human pathogens. *Vibrio mimicus* is also a human pathogen with similar microbiological characteristics as *Vibrio cholerae*. Other species have also been isolated from patients with gastroenteritis. *V. metschnikovii*, *V. fluvialis* and *V. hollisae* infections have been associated with consumption of raw or undercooked shellfish. The isolate was identified as *Vibrio mimicus*.

Davis first described this *V. mimicus* in 1981. It causes diarrhea in humans following the consumption of contaminated undercooked or raw shellfish. *Vibrio mimicus* is similar to *Vibrio cholerae* serogroup non-O1 in most of its

clinical and epidemiological aspects. It is different from *V. cholerae* in its failure to ferment sucrose. *V. mimicus* appear as small green colonies on TCBS medium but will grow on most common media without NaCl. The virulence of this organism is poorly characterized, but some isolates have the cholera toxin gene.

Table - 1 *Vibrio mimicus*			
Tests	24 hrs	48 hrs	7 days
0.129 10 mcg	Sensitive		
0.129 150 mcg	**Sensitive**		
TCBS growth	**+ Green**	-	-
NaCl 0 %	+	+	+
NaCl 3 %	+	+	+
NaCl 6 %	+	+	+
NaCl 8 %	-	-	-
NaCl 10 %	-	-	-
Indole	+		
Oxidase	**+**		
Catalase	+		
Urease	-	-	-
Dextrose *	+/No gas	+/No gas	+/No gas
Mannitol	+	+	+
Lactose	-	-	-
Sucrose	-	-	-
Arabinose	-	-	-
Trehalose	+	+	+
Salicin	-	-	-

* All carbohydrate tests performed by fermentation methods.

LabLink is published quarterly by the Michigan Department of Community Health, Bureau of Laboratories, to provide laboratory information to Michigan health professionals and the public health community.

Director, Bureau of Laboratories Frances Pouch Downes, Dr.P.H.

Editor Susan L. Shiflett